

Redox activity of urban quasi-ultrafine particles from primary and secondary sources

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ABSTRACT

To characterize the redox activity profiles of atmospheric aerosols from primary (traffic) and secondary photochemical sources, ambient quasi-ultrafine particles were collected near downtown Los Angeles in two different time periods – morning (6:00–9:00 PDT) and afternoon (11:00–14:00 PDT) in the summer of 2008. Detailed chemical analysis of the collected samples, including water-soluble elements, inorganic ions, organic species and water soluble organic carbon (WSOC) was conducted and redox activity of the samples was measured by two different assays: the dithiothreitol (DTT) and the macrophage reactive oxygen species (ROS) assays. Tracers of secondary photochemical reactions, such as sulfate and organic acids were higher (2.1 ± 0.6 times for sulfate, and up to 3 times for the organic acids) in the afternoon period. WSOC was also elevated by 2.5 ± 0.9 times in the afternoon period due to photo-oxidation of primary particles during atmospheric aging. Redox activity measured by the DTT assay was considerably higher for the samples collected during the afternoon; on the other hand, diurnal trends in the ROS-based activity were not consistent between the morning and afternoon periods. A linear regression between redox activity and various PM chemical constituents showed that the DTT assay was highly correlated with WSOC ($R^2 = 0.80$), while ROS activity was associated mostly with water soluble transition metals (Vanadium, Nickel and Cadmium; $R^2 > 0.70$). The DTT and ROS assays, which are based on the generation of different oxidizing species by chemical PM constituents, provide important information for elucidating the health risks related to PM exposure from different sources. Thus, both primary and secondary particles possess high redox activity; however, photochemical transformations of primary emissions with atmospheric aging enhance the toxicological potency of primary particles in terms of generating oxidative stress and leading to subsequent damage in cells.

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1. Introduction

Several recent toxicological studies have confirmed the redox properties of ambient particulate matter (PM) and their capability to generate reactive oxygen species (ROS) in biological systems (Donaldson et al., 2002; Xia et al., 2004). The production of these pro-oxidant species and their subsequent damage to important macromolecules (e.g., DNA, proteins, lipids) are thought to be implicated in numerous diseases including respiratory (Li et al., 2009a), cardiovascular (Delfino et al., 2005) and neurodegenerative disorders (Peters et al., 2006). Most of these studies indicate

a consistently higher redox activity of quasi-ultrafine (<150 nm) particles compared to fine and coarse particles (Cho et al., 2005; Ntziachristos et al., 2007a). Recent studies have also linked PM toxicological characteristics, and in particular redox activity, to their chemical composition, such as organic species and transition metals (Biswas et al., 2009; Hiura et al., 1999; Kumagai et al., 1997; Kunzli et al., 2006; Schaumann et al., 2004).

Atmospheric quasi-ultrafine particles (quasi-UFP) originate from two broad categories of sources: primary and secondary. Primary particles are directly emitted from combustion sources, including heavy and light duty vehicles, wood smoke, and industries including, for example, power plants. In the presence of various atmospheric oxidants, primary particles may undergo photochemical processing yielding secondary particles with distinctly different physical and chemical characteristics compared to their precursor primary particles.

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Diurnal changes in physical properties (particle number size distribution and volatility) of the quasi-ultrafine particles at an urban location near downtown Los Angeles have been discussed in detail by Moore et al. (2007). In a companion paper, the chemical composition of ambient samples collected in two different time periods (morning and afternoon) at the same site was also presented (Ning et al., 2007). Results from both studies indicated a strong influence of commute traffic emissions during the morning hours as evidenced by elevated concentrations of nitrogen oxides (NO_x), carbon monoxide (CO), black carbon (BC) (Moore et al., 2007), alkanes, polycyclic aromatic hydrocarbons (PAHs) and hopanes (Ning et al., 2007). By contrast, the afternoon concentrations of oxygenated organic acids and sulfate rose, while other species were diluted by the increased mixing height or lost due to increasing temperature. These are clear indicators that secondary photochemical reactions are a major source of quasi-ultrafine aerosols in the afternoon. The reaction mechanisms leading to the generation of secondary organic aerosols (SOA) have not been fully characterized due to the complex nature of organic compounds in the atmosphere. Recent studies indicate that the volatilization and subsequent photo-oxidation of the semi-volatile fraction of primary organic aerosols contribute substantially to the formation of SOA. This contribution far exceeds that from the photo-oxidation of primary volatile organic compounds (VOCs) (Robinson et al., 2007). The differences in the physico-chemical characteristics of primary and secondary particles will likely lead to differences in their redox activities including their ability to induce cellular oxidative stress (Baltensperger et al., 2008; Delfino et al., 2009; Ntziachristos et al., 2007a). Recent laboratory experiments also indicate that combining primary diesel particles with ozone (in order to mimic atmospheric photochemical transformation processes) substantially enhances the redox activity of the PM (Li et al., 2009b); however, there are very few studies investigating this important issue using real world atmospheric aerosols.

The present study focuses on comparing the redox activity profiles of the quasi-ultrafine particles (<180 nm in this study) at an urban site near downtown Los Angeles collected during two different time periods of the day – morning and afternoon, representing particles characterized by primary emissions and secondary aerosols formation, respectively. The redox activity of the collected particles has been measured by two independent assays: 1) the DTT assay, and 2) the macrophage ROS assay. Detailed chemical analyses of the collected samples including water-soluble elements, inorganic ions, organic species and water soluble organic carbon have been conducted to distinguish the chemical properties of the source specific particles, and also to investigate their correlation with the measured redox activities. Our goal was to contrast the toxicity profiles of ambient quasi-ultrafine particles emitted from primary vehicular sources with those produced from secondary processes and atmospheric aging.

2. Methods

2.1. Sampling location

This study was conducted at the Particle Instrumentation Unit (PIU) of Southern California Particle Center (SCPC) on the campus of University of Southern California (USC) near downtown Los Angeles. The sampling site is located approximately 130 m to the NE of the I-110 freeway, near construction, parking facilities and other nominal sources of pollutant emissions. Pollutant sources near this site are predominantly from vehicular sources with traffic patterns consistent with a mixed-use urban environment. Light duty gasoline vehicles constitute the major fraction (>93%) of traffic at the stretch of freeway near the site, with the rest being heavy duty diesel vehicles. Studies indicate that the average

monthly PM chemical composition in the quasi-ultrafine mode at the USC and other similar Los Angeles-area sites is dominated by organic carbon, which varies from 40 to 90% depending upon the season and location (Fine et al., 2004; Hughes et al., 1998; Sardar et al., 2005). Additional sampling site information including the results of earlier investigations of the physical and chemical properties of the PM observed at the USC site can be found in Ning et al. (2007) and Fine et al. (2004).

2.2. Sampling protocol

Sampling was conducted between June and August, 2008 over a period of 10 consecutive weeks (excluding weekends). Quasi-ultrafine particles ($D_p < 180$ nm) were collected using a low pressure drop impactor on Zeflur PTFE Membrane Filters (Pall Life Sciences, 3 μm , 8" \times 10", 28139-597) seated in a specially designed 20 \times 25 cm high volume sampler (HIQ Environmental Products Co., CA). The impactor has been designed primarily as a separator of quasi-ultrafine particles from accumulation mode ($0.18 \mu\text{m} \leq D_p \leq 2.5 \mu\text{m}$) particles at a flow rate of 400 L min^{-1} . The design and operating performance of the multi-slit impactor have been described in detail previously (Misra et al., 2002). Time integrated samples were collected during both "morning" (6:00–9:00 PDT) and "afternoon" (11:00–14:00 PDT) time periods and were grouped into three sample sets (S1, S2, S3). There were 18 days total for the S1 sampling set, while both the S2 and S3 sets were collected for 16 days each. As the samples were composited for each grouping period (i.e., particles were collected on the same filter); the total number of samples for the entire campaign was 6 – two samples (morning and afternoon) for each grouping period (S1, S2 and S3). Thus, each sample of set S1 encompassed 54 h (18 days multiplied by 3 h day^{-1}), while 48 h (16 days multiplied by 3 h day^{-1}) for each sample of sets S2 and S3. The "morning" period corresponds to rush hour traffic when the ambient aerosols at the sampling site are dominated by primary particles freshly emitted from vehicles on the nearby freeway (Moore et al., 2007). The "afternoon" period represents the mixture of primary and secondary particles undergoing physical and chemical changes (i.e., photo-oxidation, volatilization, dilution and possibly re-suspension) (Moore et al., 2007). The strong influence of secondary photochemical sources during the afternoon at the sampling site is evidenced by the concentration of ozone ($[\text{O}_3]$; averaged over three time periods, i.e., S1, S2 and S3), which increased from 9 ± 4 ppb in the morning period to 50 ± 10 ppb in the afternoon period. Meteorological conditions were quite consistent during the entire study period, with higher temperature and lower humidity in afternoon ($28 \pm 2^\circ\text{C}$ and $44 \pm 5\%$) compared to the morning period ($20 \pm 1^\circ\text{C}$ and $70 \pm 5\%$). The wind direction was mostly from south-west at the site. A Scanning Mobility Particle Sizer (SMPS Model 3080 and DMA Model 3081, condensation particle counter Model 3022A, TSI Inc., St. Paul, MN) was also set up concurrently to measure the particle size distribution. The sampling flow rate was 1.5 L min^{-1} , with a scan up time of 150 s. The minimum and maximum sizes detectable at these settings are 7.64 and 225 nm, respectively. The mass concentrations of quasi-ultrafine particles in both the morning and afternoon periods were obtained using a parallel MOUDI (Micro-Orifice Uniform Deposit Impactor; MSP Corporation, Minneapolis, MN) measurement. The mass concentration of quasi-ultrafine particles measured by the MOUDI and the multi-slit impactor has been demonstrated to be in agreement ($R^2 > 0.85$) (Misra et al., 2002).

2.3. Sample analysis

All filter samples, along with field blanks and lab blanks, were extracted in high purity deionized water at ambient temperature using a shaking table. The water extracts were analyzed for water

soluble organic carbon (WSOC), water soluble ions, and water soluble trace elements. Water soluble ions were analyzed using two Ion Chromatography (IC) analyses: 1) analysis for cations; and 2) analysis for anions (Zhang et al., 2008). Trace elements were analyzed from the water extracts using a high resolution Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) as described by Zhang et al. (2008). Water soluble organic carbon (WSOC) for all the samples was analyzed using a Shimadzu TOC-5000A liquid analyzer (Decesari et al., 2001). A separate section of the sample, field blank and lab blank filters was extracted with a solvent mixture of methylene chloride and methanol using Soxhlet extraction. The extracts were concentrated before gas chromatography/mass spectrometry analysis as described by Stone et al. (2008). All chemical analyses were conducted with check standards and multipoint calibration curves to assure accurate quantification. All results were blank subtracted using the field blanks processed and analyzed with the samples.

The redox activity of the collected particles was quantified by two different assays: 1) consumption of dithiothreitol in a cell-free system (DTT assay), and 2) macrophage ROS – in vitro exposure to rat Alveolar Macrophage (AM) cells using Dichlorofluorescein Diacetate (DCFH-DA) as the ROS probe. The former assay determines in quantitative terms the capacity of a test sample to transfer electrons from dithiothreitol (DTT) to oxygen – a reaction analogous to the cellular redox reaction involving NADPH and oxygen. The electron transfer is monitored by the rate at which DTT is consumed under a standardized set of conditions, and the rate is proportional to the concentration of the redox-active species in the PM sample. To assess the relevance of this assay to cellular effects, studies have been conducted in which the DTT based redox activity of a sample has been compared with its capacity to induce the stress protein, hemeoxygenase-1 in two cell lines – RAW 264.7 and BEAS-2B. RAW 264.7 is a murine macrophage cell line that mimics the oxidative stress response of pulmonary alveolar macrophages (Hiura et al., 1999; Li et al., 2002). BEAS-2B is a transformed human bronchial epithelial cell line, which mimics the oxidative stress response of primary bronchial epithelial cells (Li et al., 2002). DTT activities of PM samples were found to correlate with the induction of hemeoxygenase-1 in both cell lines (Li et al., 2003). These data suggest that redox activity measured by this procedure is a reasonable predictor of oxidative stress status in cells. The methodological procedure used for the DTT assay conducted for this work is described in detail by Cho et al. (2005).

The macrophage ROS assay is a fluorogenic cell-based method to examine the production of reactive oxygen species and is performed in rat alveolar macrophages (NR8383, American Type Culture Collection), which are exposed to aqueous suspensions of PM. The ROS probe used in this method – DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) is a non-fluorescent, membrane permeable compound. Upon entering a cell, it is de-acetylated by cellular house-keeping esterases yielding 2',7'-dichlorodihydrofluorescein (DCFH). Non-fluorescent DCFH can then be converted by free radicals and other oxidants within the cell into the highly fluorescent 2,7-dichlorofluorescein (DCH) which can be easily monitored using a fluorescence plate reader. Further detail of this assay is given in Landreman et al. (2008). High purity deionized water was used for the filter extraction technique for both the DTT and ROS assays.

3. Results and discussion

3.1. Physical parameters

Fig. 1a shows the hourly average particle number concentration in the size range from 7.64 nm to 180 nm, measured by the SMPS during the sampling campaign. As shown in the figure, the diurnal

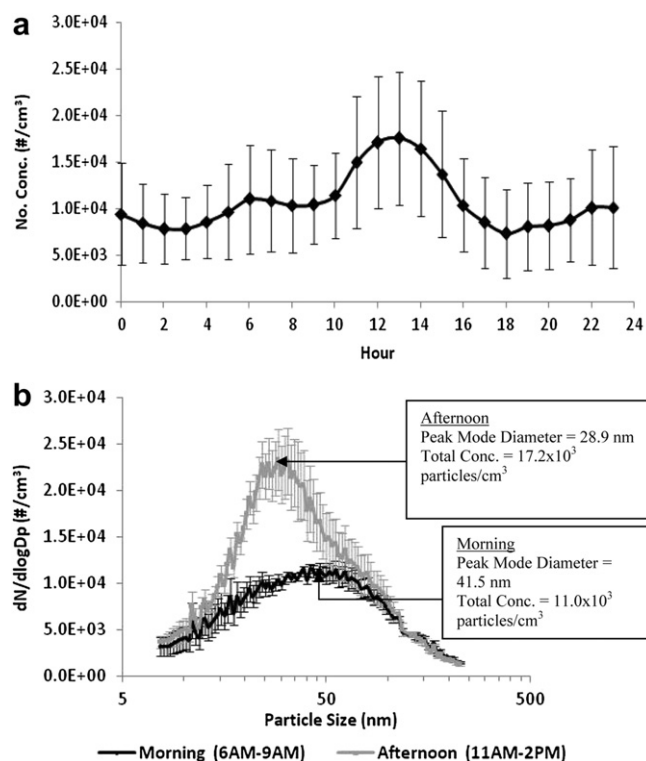


Fig. 1. a) Diurnal profile of the average particle number concentration of quasi-ultrafine particles measured at the USC sampling site and (b) the average particle number size distribution of quasi-ultrafine particles in "morning" and "afternoon" periods.

profile displays a shoulder in the morning (6:00–9:00 AM) to the distinguishable peak in the afternoon (11:00 AM to 2:00 PM). This represents emissions from morning rush hour traffic and secondary photochemical reactions occurring in the afternoon at the site, respectively (Moore et al., 2007). Average particle number concentrations in the afternoon are 1.6 ± 0.1 times those in the morning, suggesting secondary particle formation consistent with the observations by Moore et al. (2007) at the same site. Fig. 1b shows the average particle number size distribution ($dN/d\log D_p$) measured during the morning and afternoon periods, respectively. The size distributions in both the morning and afternoon are monomodal; however, the afternoon period is distinguished by a smaller peak mode diameter (28.9 ± 1.1 nm) compared to the morning period (peak mode diameter = 41.5 ± 1.5 nm). The larger peak mode diameter in the morning period may be due to the combined effects of condensation of organic vapors co-emitted from traffic sources onto freshly emitted ultrafine PM, which would be result of the lower temperatures and atmospheric dilution that prevail during this time period, and possibly to coagulation of highly concentrated nanoparticles. Both of these processes will enhance the growth of the particles emitted from vehicular sources compared to the relatively freshly formed secondary aerosols in the afternoon period (Zhu et al., 2002). Average mass concentrations of quasi-ultrafine particles were also higher in the afternoon period ($5.7 \pm 2.6 \mu\text{g m}^{-3}$) compared to $3.8 \pm 1.7 \mu\text{g m}^{-3}$ in the morning period, indicating the additional contribution of secondary aerosol formation to the total PM_{0.18} (particles with aerodynamic diameter ≤ 180 nm) mass.

3.2. Chemical parameters

Fig. 2 shows the average mass concentration of major water soluble inorganic ions [sulfate (SO_4^{2-}), ammonium (NH_4^+), and nitrate

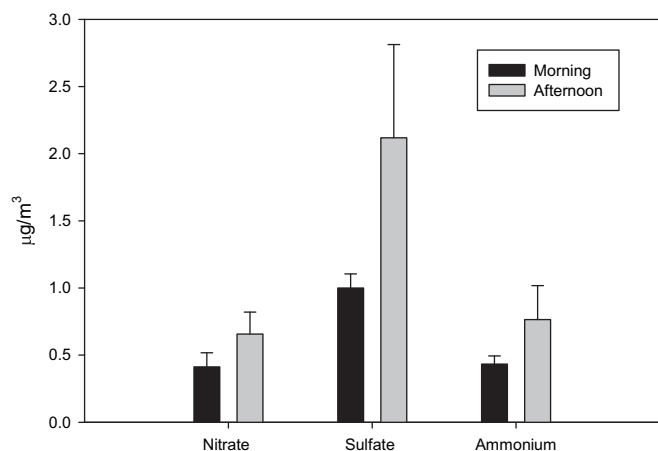


Fig. 2. Average concentration of the major inorganic ions in quasi-ultrafine particles collected in the morning and afternoon periods at the USC sampling site.

(NO_3^-) in quasi-ultrafine particles measured at the sampling site in the morning and afternoon periods. Concentrations of these ionic species are higher in the afternoon period compared to those in the morning, with afternoon-to-morning ratios of 1.76 ± 0.38 , 1.60 ± 0.04 and 2.11 ± 0.61 for NH_4^+ , NO_3^- and SO_4^{2-} , respectively. This increase in concentrations in the afternoon period may be attributed to the oxidation of precursor gases (e.g., ammonia, sulfur dioxide and nitric oxide) by photochemical byproducts [e.g., ozone (O_3), hydroxyl free radical (HO^\bullet), hydroperoxyl radical (HO_2^\bullet)] followed by nucleation and/or condensation onto pre-existing particles (Ning et al., 2007). Following the approach by Zhang et al. (2004a), particle acidity can be expressed as the ratio of sum of measured ammonium and sodium concentrations to the concentrations needed to fully neutralize the measured sulfate, nitrate and chloride, with a value of unity suggesting the full neutralization and a value close to zero suggesting an acidic aerosol. The ratios measured during the morning and afternoon periods are 0.93 ± 0.03 and 0.82 ± 0.01 , respectively, thereby indicating an $11 \pm 3\%$ increase in particle acidity in the afternoon period. This is consistent with the previous studies demonstrating higher acidity of the ultrafine particles [measured to fully neutralizing ammonium ratio of ~ 0.80 ; (Zhang

et al., 2004a)] in the initial stage of photochemically induced nucleation.

Table 1 shows the comparison of the mass concentrations (average and standard deviation) of water-soluble trace elements and metals in the quasi-ultrafine particles in the morning and afternoon. A total of twenty elements have been quantified as shown in the table. Sulfur (S), Aluminum (Al), Calcium (Ca), Iron (Fe) and Sodium (Na) are the dominant species in the ambient atmosphere both in the morning and afternoon periods. The concentration of certain metals [Vanadium (V), Chromium (Cr), Nickel (Ni), Copper (Cu), Arsenic (As), Cadmium (Cd) and Zinc (Zn)] was higher in the morning period. These heavy metals are known to be emitted from vehicles from both fuel combustion and lube oil emissions (Geller et al., 2006). Cu originates from vehicle brake abrasion (Garg et al., 2000; Sanders et al., 2003; Sternbeck et al., 2002) and Zn is mostly a product of tire attrition (Singh et al., 2002). Elevated levels of these metals in the morning period suggest traffic as the primary source of particles during the morning hours at the sampling site. Other metals such as Na, Magnesium (Mg), Phosphorus (P), Al, Ca, Potassium (K), Titanium (Ti), Manganese (Mn), Fe, Cobalt (Co) and Barium (Ba) displayed a different pattern with higher values in the afternoon period. These metals also exhibit a high degree of correlation amongst themselves (see Table S2 in the supplementary information; $R > 0.70$) and probably originate from re-suspension of road dust, which is higher in the afternoon due to the higher wind speed ($3.5 \pm 0.3 \text{ m s}^{-1}$ compared to $0.9 \pm 0.4 \text{ m s}^{-1}$ in the morning period), lower relative humidity ($44 \pm 5\%$ compared to $70 \pm 5\%$ in the morning period) and possibly higher vehicle speeds on the nearby streets and freeways, compared to the congested morning traffic hours. Although, the majority of these species are expected to be partitioned in the coarse PM ($\text{PM}_{10-2.5}$), there is a significant fraction in the sub-200 nm range, as demonstrated by earlier investigations in urban areas (Garg et al., 2000; Ntziachristos et al., 2007b).

Fig. 3a shows the average mass concentration of quasi-UFP WSOC and CO in the morning and afternoon periods. CO is an inert gas species often used as a marker of vehicular emissions (Zhu et al., 2002) and atmospheric dilution (Westerdahl et al., 2009), while WSOC consists of a variety of polar organic compounds and is regarded as an indicator of secondary organic aerosols (SOA) (Docherty et al., 2008). As shown in the figure, the CO concentration

Table 1

Average concentrations of water-soluble elements in ambient quasi-ultrafine particles measured by ICP-MS at the USC sampling site during morning and afternoon periods.

Metal	Morning		Afternoon		Ratio (morning/afternoon)
	Average Conc., ng m^{-3}	S.D.	Average Conc., ng m^{-3}	S.D.	
Na	57.82	24.98	195.59	47.54	0.30
Mg	17.65	8.16	64.36	6.93	0.27
Al	112.55	42.20	311.37	34.95	0.36
P	3.81	6.00	10.44	3.36	0.36
Ca	110.31	127.09	198.15	50.86	0.56
S	472.53	311.01	578.42	130.74	0.82
K	22.39	17.77	82.59	34.39	0.27
Ti	7.02	3.36	24.43	6.77	0.29
V	5.78	4.72	5.50	1.43	1.05
Cr	1.35	1.24	0.55	0.49	2.46
Mn	2.34	1.30	4.73	1.19	0.49
Fe	106.94	62.39	286.21	78.88	0.37
Co	0.09	0.05	0.18	0.03	0.49
Ni	3.09	2.46	2.12	0.75	1.46
Cu	16.26	18.61	10.75	6.13	1.51
Zn	20.26	13.06	14.87	4.42	1.36
As	0.22	0.13	0.19	0.07	1.15
Cd	0.06	0.04	0.05	0.01	1.21
Ba	3.06	1.68	10.49	0.87	0.29
Pb	3.72	2.83	4.38	1.61	0.85

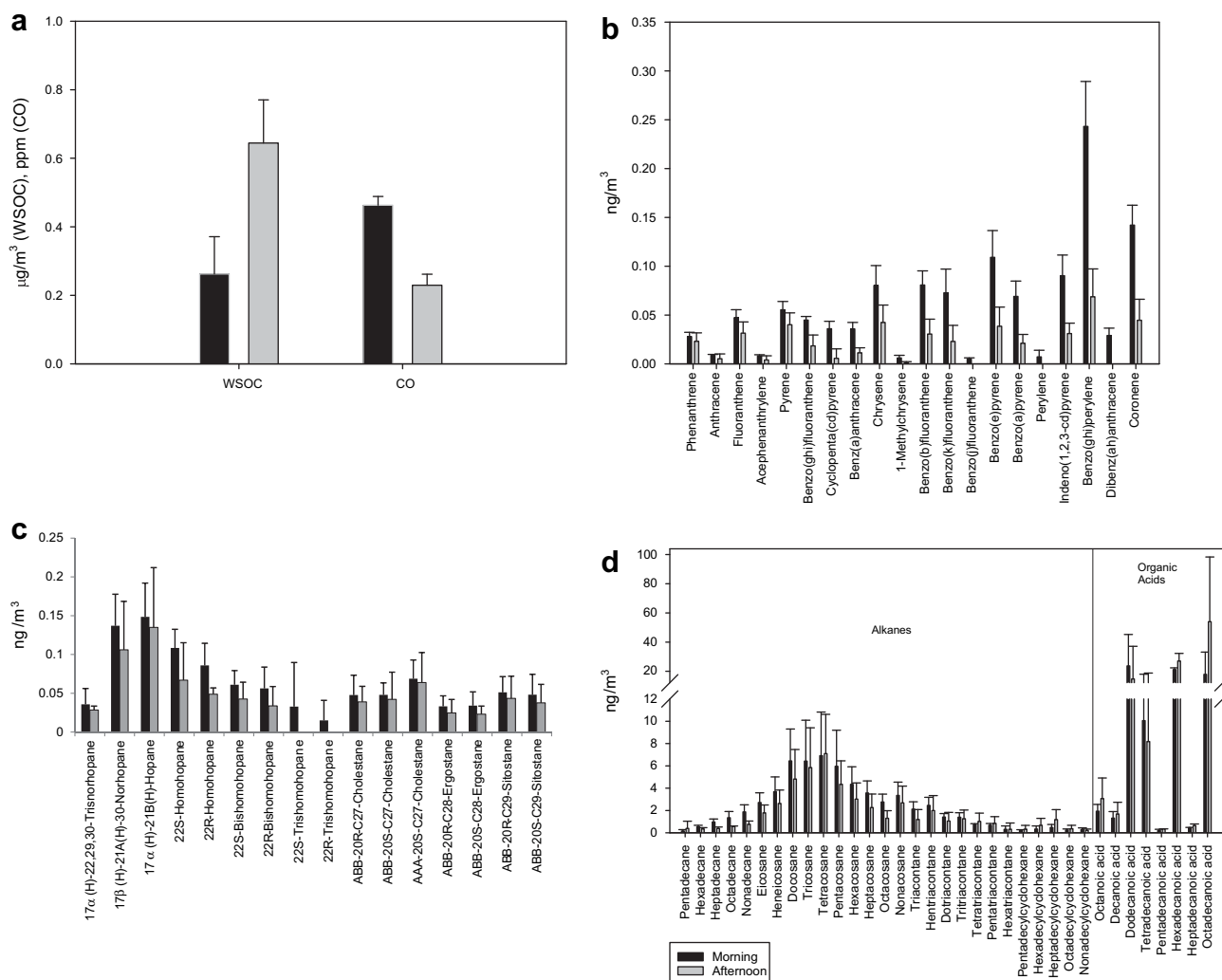


Fig. 3. Average concentration of the various organic species measured at the USC sampling site during the morning and afternoon periods a) water soluble organic carbon and carbon monoxide, b) polycyclic aromatic hydrocarbons (PAHs), c) hopanes and steranes, and d) alkanes and organic acids.

decreased by a factor of 2.0 ± 0.1 in the afternoon, as a result of a decrease in vehicular emissions coupled with an increase in the mixing height and atmospheric dilution in the afternoon period. However, despite the 2-fold increase in atmospheric dilution suggested by the CO concentrations, the average concentration of WSOC was elevated by 2.5 ± 0.9 times compared to the morning period. We interpret this to indicate the formation of SOA driven by the oxygenation of particles with aging in the atmosphere during photochemical reactions in the afternoon.

Fig. 3 also shows the average quasi-UFP mass concentrations of speciated organic compounds [PAHs, (Fig. 3b); hopanes and steranes, (Fig. 3c); and alkanes and organic acids, (Fig. 3d)] in the morning and afternoon periods. Most of the PAHs, hopanes, steranes, and alkanes were higher in the morning period compared to the afternoon period, attributed to their origin from vehicular sources (Ning et al., 2007). However, morning-to-afternoon ratio for certain species (particularly PAHs and alkanes) is relatively higher [up to 3.5 ± 0.9 as for benzo (GHI) perylene], compared to that for CO (morning-to-afternoon ratio = 2.0 ± 0.1 ; Fig. 3a). The relatively larger decrease in the concentration of PAHs and alkanes compared to CO in the afternoon indicates their possible volatilization and photo-oxidation in addition to dilution. Interestingly, in the fine size range ($PM_{2.5}$), concentrations of these organic species

(PAHs and alkanes) were higher in the afternoon than the morning period (Table S1 in supplementary information). We hypothesize that volatilization of these species from the ultrafine particles in afternoon period and subsequent re-condensation onto the larger particles might be responsible for this trend (Venkataraman et al., 1999). Similar observations on the partitioning of PAHs between ultrafine and accumulation PM modes with increasing ambient temperature have also been made in other studies (Miguel and Friedlander, 1978; Pierce and Katz, 1975).

By contrast, concentrations of most of the organic acids were higher in the afternoon period (afternoon-to-morning ratio ranges from 1.2 to 3.0). These acids are probably the products of photochemical oxidation of either organic gases or semi-volatile species evaporating from the primary particles (Zhang et al., 2004b). Food cooking and vegetative detritus emissions might be included into other sources of some organic acids, but the sampling site is not substantially impacted by these sources, which supports the notion that atmospheric chemistry is likely responsible for the increased concentrations of these acids in the afternoon. The afternoon-to-morning ratio was higher (up to 3) for high carbon number organic acids (C_{15} – C_{29} , e.g., Octadecanoic Acid, Pentadecanoic Acid) compared to the low carbon number acids (e.g., Octanoic Acid, Decanoic Acid; ratio ~ 1.5), probably due to the higher volatility of

organic species associated with decrease in carbon number (Ning et al., 2007).

3.3. Redox activity

The redox activity of the quasi-ultrafine particles was measured by two different methods as described earlier – the DTT and macrophage ROS assays. Fig. 4 shows the results of the DTT assay for the three samples sets (S1, S2, and S3) and their average values in the morning and afternoon periods. The results have been expressed as mass (of PM) based activity (Fig. 4a) in $\text{nmol min}^{-1} \mu\text{g}^{-1}$ and also volume (of air) based activity (Fig. 4b) in $\text{nmol min}^{-1} \text{m}^{-3}$, respectively. As shown in Fig. 4a, the mass-based DTT activity of the quasi-UFP is consistently higher in the afternoon period for all of the sample sets with an average afternoon-to-morning ratio of 1.8 ± 0.7 . The DTT assay measures the capability of particles to generate superoxide radicals ($\text{O}_2^{\bullet-}$) in their interaction with the thiol group compounds, which are the main antioxidants present in endothelial cells (Foresti et al., 1997). Although this assay is insensitive to hydroxyl free radicals (HO^\bullet), $\text{O}_2^{\bullet-}$ produced in the above reaction can play a role in the generation of HO^\bullet also, by regenerating the precursors of Fenton's reaction (e.g., Fe^{+2} and H_2O_2) (Schoonen et al., 2006). In a recent experimental study, Li et al. (2009b) demonstrated higher redox activity measured by the

DTT assay of diesel exhaust particles (DEPs) aged with O_3 compared to the freshly emitted diesel PM. Our results showing the increased DTT activity of the quasi-ultrafine particles in afternoon period are very consistent with the work described earlier by Li et al. (2009b), and lead to one of the most important findings of this study – compounds associated with secondary aerosols produced by photochemical processes in the afternoon period possess higher redox activity, and thus are more capable of generating free radicals and causing cell damage in biological systems.

As shown in Fig. 4b, the DTT activity m^{-3} of air is also consistently higher for the afternoon samples, with an average afternoon-to-morning ratio of 2.8 ± 1.1 (compared to 1.8 ± 0.7 for the mass-based activity). The difference between the volume based and mass-based DTT activity may have important implications with respect to the assessment of public exposure and the relative PM toxicity. While the mass-based redox activity is appropriate for comparing the relative PM toxicity from different sources, the volume-based activities become important in the context of the overall public exposure and the associated risks of individual contributions from various sources to ambient PM concentrations.

Fig. 5 shows the results of macrophage ROS assay in the morning and afternoon periods, expressed as μg of Zymosan units per mg of PM mass (a), and per m^3 of air (b) on the collected quasi-UFP. There

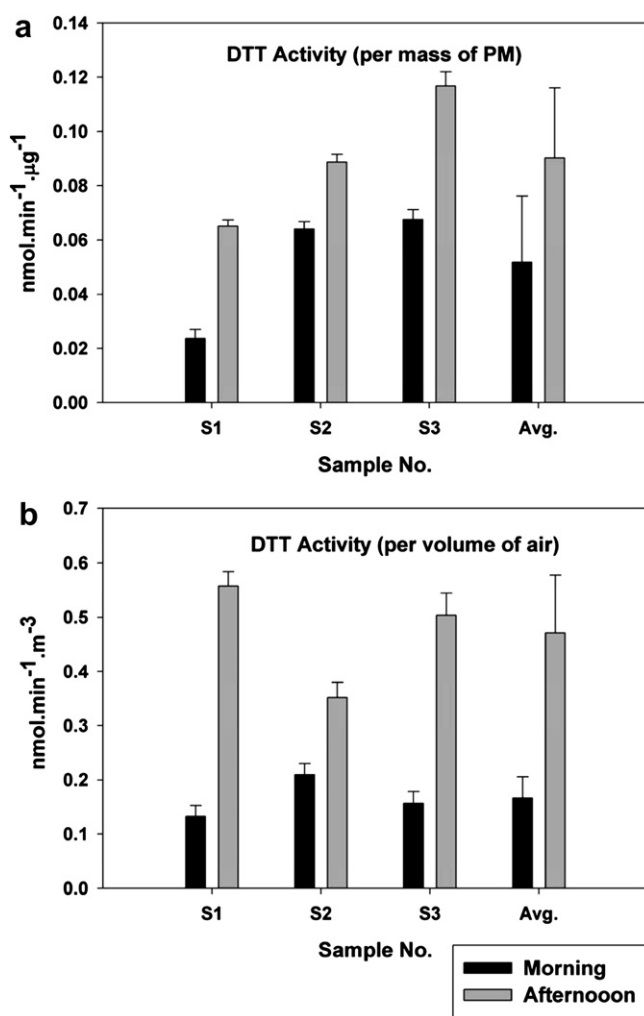


Fig. 4. Redox activity (expressed as nmol of DTT min^{-1}) of quasi-ultrafine particles collected at the USC sampling site during the morning and afternoon periods; a) per μg of particulate mass and b) per m^3 of volume of air.

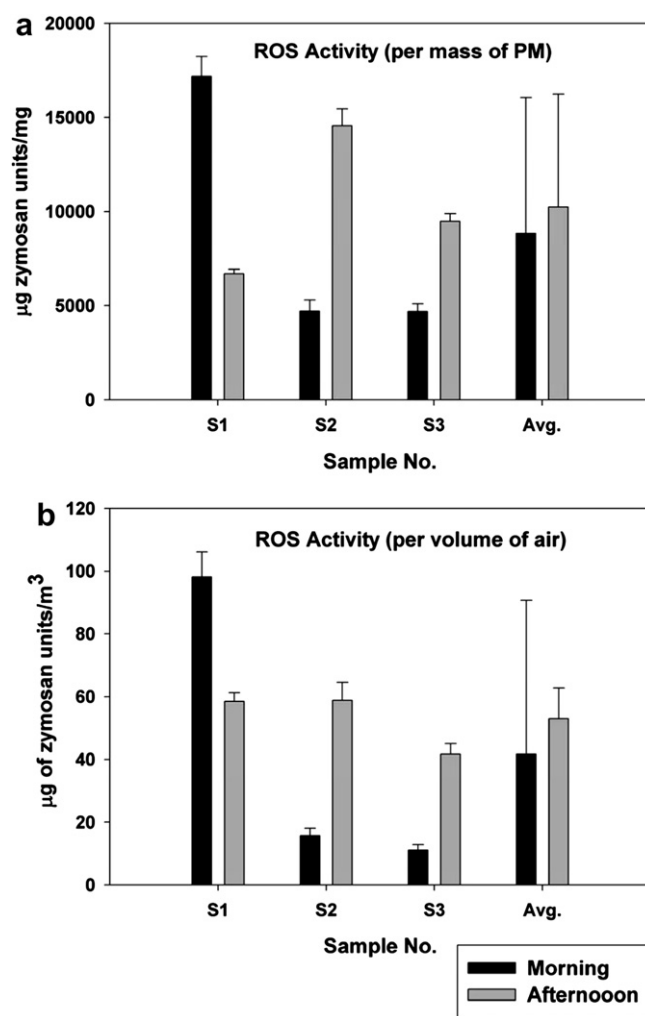


Fig. 5. Redox activity (expressed as the ROS response, or μg of Zymosan units) of quasi-ultrafine particles collected at the USC sampling site during the morning and afternoon periods; a) per mg of particulate mass and b) per m^3 of volume of air.

was significant variation in the ROS activity for the three sample sets (S1, S2 and S3) and the afternoon-to-morning ratio averaged over the three grouped periods was 1.2 ± 1.1 . Although the ROS activity of sample sets, S2 and S3 was higher in afternoon period, very high activity was observed for the morning period of sample set S1. The macrophage ROS assay measures the generation of reactive oxygen species by its reaction with DCFH. The ROS probe used in this method is sensitive to a broad spectrum of oxidants including HO[•], hydrogen peroxide (H₂O₂) and nitric oxides (NO[•]) (Schoonen et al., 2006). The inconsistent trend in ROS activity for the morning and afternoon samples indicates that, although secondary organic compounds formed in afternoon period may contribute to the formation of HO[•] in biological cells, the overall capability of PM to generate free radicals is also dependent on species emitted from primary sources in the morning period. The following section will further elaborate on the relationship between the PM chemical constituents and free radicals generation.

3.4. Correlation of redox activity with chemical constituents of PM

The DTT and ROS assays measure the PM redox activity based on the generation of different reactive oxygen species. The dissimilar diurnal profiles of PM activity obtained by these assays indicate different pathways of redox active chemical PM species in generating cellular oxidative stress. Linear regression analysis was carried out to investigate the association of the DTT and ROS activities with quasi-UFP chemical composition. Table 2 shows a summary of the regression analysis [i.e., slope, intercept, correlation coefficients (R^2) and the associated levels of significance (p value)] for select species which were correlated ($R^2 > 0.60$) with either the DTT or ROS rates. As shown in the table, we observed high correlation between WSOC and the DTT activity of quasi-UFP ($R^2 = 0.82$; $p = 0.01$), despite the rather limited number of data points. Previous studies have also documented the positive correlation of DTT activity and WSOC content of the PM (Biswas et al., 2009; Hu et al., 2008; Verma et al., 2009). This supports our hypothesis that the increased solubility of organic compounds mediated by photochemical oxidants during secondary organic aerosols formation enhances the capability of PM to generate free radicals.

The measured organic compounds were further categorized as per different functional groups (PAHs, hopanes and steranes, alkanes, high and low carbon number organic acids) to investigate their correlation with redox activity. As shown in Table 2, DTT activity is positively correlated with high carbon number organic acids (C₁₅–C₂₉) ($R^2 = 0.71$; slope = 0.01). Many of these organic acids are formed by the photo-oxidation of semi-volatile organic species, producing polar oxygenated compounds. Thus their positive correlation with DTT suggests their partial contribution to the association of DTT with WSOC discussed earlier. However, low carbon number organic acids (C₉–C₁₄) shows negative correlation ($R = -0.50$; Table S2 in supplemental information) with DTT. This is likely due to their higher volatility, resulting in their decreased concentrations in the afternoon and thus limiting their contribution to WSOC.

In the present study, the DTT activity of the ultrafine particles is observed to be negatively correlated with measured PAHs ($R^2 = 0.68$, slope = -0.33) as shown in Table 2. This is in contrast to previous studies showing rather modest to strong positive associations between DTT activity and PAHs (Cho et al., 2005; Ntziachristos et al., 2007a). Although PAHs themselves do not contain the functional groups capable of catalyzing the oxidation of DTT, their possible photo-oxidation during photochemical episodes in the afternoon period may convert them to oxy-PAHs, quinones and nitro-PAHs, which are all active in DTT assay (Cho et al., 2005). However, we did not quantify these oxygenated products in the present study. Nevertheless, the negative correlation of the measured PAHs with DTT activity may be explained by their lowered concentrations in the afternoon due to possible volatilization. It is important to note that this volatilization may be followed by photo-oxidation to form oxygenated PAHs, which contribute to the increase of DTT activity in the afternoon. The difference in the sampling protocols between our study and previous studies (Cho et al., 2005; Ntziachristos et al., 2007a) might explain the contrasting correlations. In the present study, the sampling protocol was intentionally designed to distinguish the impacts of primary and secondary sources on PM toxicity. This differs from the uninterrupted time integrated sampling of previous studies, ranging from several hours to several days, without discriminating the morning and afternoon periods, which would obscure the impact of

Table 2
Summary of the regression analysis [slope, intercept, correlation coefficients (R^2) and the associated levels of significance (p value)] for select species with DTT and ROS levels. Species included in this table are correlated ($R^2 > 0.60$, shown bolded) with at least one of the assays.

Species	DTT Activity				Macrophage ROS			
	R^2	p	Slope ^a	Intercept ^b	R^2	p	Slope ^a	Intercept ^b
NO ₃ ⁻	0.68	0.04	0.82	-8.12	0.20	0.37	79.97	4.57
SO ₄ ²⁻	0.69	0.04	0.0	0.01	0.08	0.59	11.88	28.79
NH ₄ ⁺	0.62	0.07	0.58	-0.03	0.14	0.47	48.41	18.32
WSOC	0.82	0.01	0.70	0.010	0.09	0.58	40.11	29.14
Mg	0.80	0.02	0.01	0.07	0.15	0.44	0.48	27.75
Al	0.64	0.06	1.00E-03	0.05	0.14	0.47	0.10	25.36
Ca	0.17	0.41	0.00	0.20	0.84	0.01	0.30	1.45
S	0.04	0.72	0.00	0.24	0.91	0.01	0.14	-25.79
K	0.71	0.04	3.00E-03	0.12	0.22	0.35	0.37	28.11
Ti	0.79	0.02	0.02	0.08	0.15	0.44	1.18	28.72
V	0.01	0.84	-0.01	0.35	0.91	0.00	9.83	-8.16
Fe	0.69	0.04	1.00E-03	0.07	0.28	0.28	0.15	18.76
Ni	0.13	0.49	-0.04	0.42	0.73	0.03	16.12	5.38
As	0.01	0.85	-0.20	0.36	0.66	0.05	281.00	-10.08
Cd	0.08	0.60	-1.82	0.42	0.77	0.02	1024.00	-9.31
Ba	0.75	0.03	0.04	0.07	0.19	0.38	3.35	24.64
PAHs	0.68	0.04	-0.33	0.59	0.02	0.79	11.02	38.33
Organic acids (C ₁₅ –C ₂₉)	0.71	0.15	0.01	0.07	0.07	0.74	0.45	44.02

Sample size for the regression analysis is $N = 6$.

^a Expressed as redox activity (nmol min⁻¹ for DTT and µg Zymosan units for ROS) per µg or ng of species (µg for inorganic ions and WSOC; ng for metals, PAHs and organic acids).

^b Expressed as nmol min⁻³ for DTT and µg of Zymosan units m⁻³ for macrophage ROS.

PAHs chemical transformation on redox properties of PM. Thus, the positive correlation in those studies may be attributed to the confounding relationship between PAHs and oxygenated PAHs. Moreover we did not observe any significant associations between DTT activity and the concentrations of hopanes, steranes and alkanes (Table S2 in supplementary information), which again suggest that organic compounds present in relatively fresh traffic PM emissions are not as redox active as their aged photo-oxidized products.

Inorganic ions such as nitrate (NO_3^-), sulfate (SO_4^{2-}) and ammonium (NH_4^+) and certain water soluble trace elements (Mg, Al, K, Ti, Fe and Ba) are also correlated with DTT activity as shown in Table 2, although none of these species is mechanistically active in this assay (Cho et al., 2005). Table S2 in the supplementary information shows that these inorganic species are correlated with WSOC ($R > 0.75$), suggesting their co-linearity with the redox active water soluble organic compounds, rather than actual contribution to DTT activity.

Table 2 shows that ROS measured PM activity is correlated ($R^2 > 0.65$; $p < 0.05$) with certain water-soluble elements such as V, Ni, Cd (transition metals) and As. V and Ni are the metals which participate in Fenton chemistry and are capable of generating hydroxyl radicals once coming in contact with the biological cells (Schoonen et al., 2006). These hydroxyl radicals can effectively oxidize the DCFH probe used in the ROS assay. The ROS activity was significantly correlated with V concentrations ($R^2 = 0.91$; $p = 0.01$), and also Ni ($R^2 = 0.73$, $p = 0.03$). Both of these species are mostly emitted from bunker fuel (heavy fuel oil) combustion, although V is emitted at a proportionally higher rate (Arhami et al., 2009). Hu et al. (2008) also observed an association between V and ROS activity in a comparison of the redox activity profiles of PM in the Los Angeles-Long Beach Harbor area and attributed this association to the influence of ship emissions on ambient PM. The significant correlation of V with the ROS activity measured in this study, in agreement with previous studies (Hu et al., 2008; Verma et al., 2009), is of particular note because it confirms the impact of heavy fuel oil combustion on particle redox activity. The remaining correlated metals (Cd and As) are associated with primary sources such as vehicular exhaust (Arhami et al., 2009; Thomaidis et al., 2003), and are unaffected by the secondary photochemical reactions occurring in afternoon.

We did not observe any significant correlation between WSOC and ROS activity ($R^2 = 0.09$; $p = 0.58$), in contrast to the case of DTT activity. It indicates that despite the possible contribution of secondary organic compounds on HO^\bullet production, the cellular generation of oxidants (and thus ROS activity) is predominantly limited by the transition metals, which are the active participants in the ROS assay and are mostly emitted from primary sources. Consequently, the relative fractions of metals and WSOC in PM determine the net change of ROS activity of particles attributable to primary and secondary sources and probably explain its inconsistent trend in the morning and afternoon period, as mentioned in previous section.

There are no studies in recent literature to document the redox activity of species such as Ca and S, which are also significantly correlated with the ROS assay results, as shown in the Table 2. Their correlation with ROS activity is probably due to their correlation with redox active transition metals (as shown in Table S2 in supplementary information).

4. Summary and conclusion

The DTT and ROS assays used in this study are two independent and intrinsically different types of analyses, likely driven by different particle components, and in that respect, they should be considered complementary to each other. Collectively, these two

assays provide an important framework for designing an effective methodology to understand the mechanism of PM toxicity. The variation in redox activity of the PM originating from different sources (i.e., primary and secondary) measured by these assays is helpful in linking PM toxicity to its source specific composition. While the ROS measured activity is mostly driven by transition metals and possibly amplified by WSOC, its overall value depends on the relative PM mass properties of both groups of species in the morning and afternoon periods. On the other hand, the DTT activity, determined primarily by WSOC, clearly increases in the afternoon period. Although primary particles have the capability of generating free radicals in cells, their photo-oxidation products – secondary particles appear to be more potent in terms of generating oxidative stress, which underscores the importance of chemical transformations of primary emissions with atmospheric aging on the overall PM toxicity and associated health risks.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.atmosenv.2009.09.019.

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